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ATTORNEY'S DOCKET NUMBER: WINTE 045244

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

SEP 18 2000

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/646740

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/01860	19 March 1999	19 March 1998

TITLE OF INVENTION

UTILIZATION OF EXTRACTS FROM IRIS PLANTS, CIMICIFUGA RACEMOSA AND TECTORIGENIN AS AN ESTROGEN-LIKE ORGAN-SELECTIVE MEDICAMENT WITHOUT UTEROTROPIC EFFECTS

APPLICANT(S) FOR DO/EO/US

Wolfgang Wuttke, Hubertus Jarry, Volker Christoffel, Barbara Spengler and Michael Popp

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☒ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Assignment Cover Sheet;
Certificate Under 37 CFR 3.37(b) Establishing Right of Assignee to Prosecute;
Notice of Change of Attorney Address; and
return postcard.

Certificate of Mailing by Express Mail
Express Mail Mailing Label Number EL243343944US Date of Deposit Sept. 18, 2000
I hereby certify that this paper or fee is being deposited with the U.S. Postal Service "Express Mail Post Office To Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Box PCT Assistant Commissioner for Patents Washington, D.C. 20231.

Luis Hernandez Perez 9/18/2000
Luis Hernandez Perez Date

PCTUS1/REV03

430 Rec'd PCT/PTO 18 SEP 2000

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/646740	PCT/EP99/01860	WINTE 045244

21. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$970.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$840.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$690.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$670.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$840.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	16 - 20 =	0	x $18.00	$0.00
Independent claims	4 - 3 =	1	x $78.00	$78.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
TOTAL OF ABOVE CALCULATIONS			=	$918.00
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	$0.00
SUBTOTAL			=	$918.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00
TOTAL NATIONAL FEE			=	$918.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☒	$40.00
TOTAL FEES ENCLOSED			=	$958.00
			Amount to be: refunded	$
			charged	$

☒ A check in the amount of **$958.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **16-2460** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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SIGNATURE

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36,045
REGISTRATION NUMBER

September 18, 2000
DATE

WINTE 045244

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the application of:

Wolfgang Wuttke, et al.

Serial No.:

Filed:

For: UTILIZATION OF EXTRACTS FROM IRIS PLANTS, CIMICIFUGA RACEMOSA AND TECTORIGENIN AS AN ESTROGEN-LIKE ORGAN-SELECTIVE MEDICAMENT WITHOUT UTEROTROPIC EFFECTS

Int. Applic. No. PCT/EP99/01860

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Group Art Unit:

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I hereby certify that this paper or fee is being deposited with the U.S. Postal Service "Express Mail Post Office To Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to: Box PCT Assistant Commissioner for Patents
Washington, D.C. 20231

Luis Hernandez Herviarez 9/18/2000
Luis Hernandez Herviarez Date

PRELIMINARY AMENDMENT

Box: Patent Application
Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Please amend claims 5-7, as follows:

5. (Amended) Use in accordance with **[any one of claims]** claim 1 **[to 3]**, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of cardiovascular diseases, particularly atherosclerosis.

6. (Amended) Use in accordance with **[any one of claims]** claim 1 **[to 3]**, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of osteoporosis.

7. (Amended) Use in accordance with **[any one of claims]** claim 1 **[to 4]**, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of climacteric disorders, particularly for preventing or alleviating hot flushes.

Please add new claims 10-16, as follows:

10. (new) Use in accordance with claim 2, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of cardiovascular diseases, particularly atherosclerosis.

11. (new) Use in accordance with claim 3, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of cardiovascular diseases, particularly atherosclerosis.

12. (new) Use in accordance with claim 2, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of osteoporosis.

13. (new) Use in accordance with claim 3, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of osteoporosis.

14. (new) Use in accordance with any one of claim 2, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of climacteric disorders, particularly for preventing or alleviating hot flushes.

15. (new) Use in accordance with any one of claim 3, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of climacteric disorders, particularly for preventing or alleviating hot flushes.

16. (new) Use in accordance with any one of claim 4, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of climacteric disorders, particularly for preventing or alleviating hot flushes.

Remarks

Prior to examination of the instant application, it is requested that the above amendments be entered. The amendments are made solely for the purpose of eliminating multiple dependencies from the claims. In view thereof, claims 1-16 will be pending in the instant application.

Respectfully submitted,
PRETTY & SCHROEDER

Dated: September 18, 2000

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Description

Use of extracts from Iridaceae and Cimicifuga racemosa and of tectorigenin as an estrogen-type, organoselective medicament having no uterotrophic effect

The present invention relates to the use of extracts from Iridaceae in accordance with claim 1, and those from Cimicifuga racemosa as an estrogen-type, organoselective medicament, and tectorigenin and/or tectorigenin glycosides as a medicament in accordance with claim 8.

17-ß-estradiol, which is formed in the ovaries (whenever estradiol is mentioned hereinafter, this always refers to physiological 17-ß-estradiol) [hereinafter also referred to as E_2], generally has a proliferation-promoting effect in the organism. Apart from controlling the female cycle, it i.a. has a homeostatic influence on the metabolism of the bone and prevents the formation of atherotic plaques at the endothelium of the vessels.

During menopause, lowering of the estradiol level takes place due to cessation of the ovarial function. This results in a weakening of proliferative processes, and in the hypothalamus results in an intensified activity of the GnRH impulse generator. (The gonadotropin-releasing hormone impulse generator is a timer in the hypothalamus, as it were, and times the pulsatile LH secretion, with steroids influencing amplitude and frequency.) In climacteric women, the resulting, stimulated LH secretion brings about the so-called "hot flushes" which are felt to be disturbing.

In the absence of sufficiently high estradiol levels in the blood, osteoclast activity and thus destruction of the bone mass is predominant, accompanied by an increased risk of skeleton breakage. At the same time, there is in the long term a risk of plaque formation in the vascular system and thus an increased risk of infarctions.

Extracts from Cimicifuga racemosa and from Belamcanda sinensis are both known from popular medicine to be capable of alleviating peri-

menopausal and post-menopausal disorders. Hitherto this has been explained through the fact that the extracts of both plant drugs exhibit an estrogen-type effect with all the positive effects thereof on a multiplicity of organs of the human body, particularly the brain, ovaries, bones, vascular system. Estrogen-type effects on uterus, vagina, breast tissue and liver would in turn be disadvantagous. What is undesirable, however, is that up to the present, a medicament from these plant drugs which might be used for organoselective prophylaxis or therapy in cases of estrogen deficiency, has not been available in the prior art.

Starting out from this state of the art, it is therefore object of the present invention to furnish plant medicaments with an estrogen-type effect, the effect of which is organoselective with no effect or only a slight effect on the uterus.

This object is independently achieved through the features of claim 1 with respect to the use of extracts from Iridaceae, through the use of extracts from Cimicifuga racemosa in accordance with claim 3. The above object is moreover achieved by the features of claim 2 with respect to a medicament on the basis of tectorigenin and/or its glycosides in accordance with claim 8.

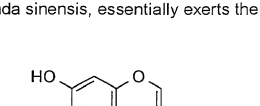
Another independent solution is represented by a plant extract containing tectorigenin and/or tectorigenin glycoside or enriched with tectorigenin and/or tectorigenin glycoside, in accordance with claim 11.

Both in in-vitro and in-vivo experiments it was surprisingly found that extracts produced both from Iridaceae, particularly Belamcanda sinensis, and from Cimicifuga racemosa with organic solvents or with supercritical CO_2 organoselectively act on the central nervous system, the bone system and the vascular system, with an effect on the uterus - the so-called uterotrophic effect - not existing. The extracts used in accordance with the invention are thus suited for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of osteoporosis.

They are moreover suited for production of a ready-formulated medicament for the selective treatment and/or prophylaxis of cardiovascular diseases, particularly of atherosclerosis.

They are moreover suited for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of peri-menopausal and post-menopausal psychovegetative disorders such as, e.g., hot flushes.

It was moreover found that the component tectorigenin, which was isolated from Belamcanda sinensis, essentially exerts the same effects as the whole extract.



Tectorigenin

This component is also found, besides Belamcanda sinensis, in other Iridaceae such as, e.g., Iris germanica, I. tectorum, I. illyrica, I. dichotoma.

Taxonomically speaking, Belamcanda sinensis is classified as follows:

Order	Liliales
Family	Iridaceae
Genus	Belamcanda
Species	Belamcanda sinensis (Leman) DC. = Pardanthus chinensis (L.) Ker-Gawler, also: Ixia chinensis L. (=Gemmingia chinensis (L.) O. Kuntze)

Preferably rhizomes, stalks, leaves and/or petals of the plants are used for producing the extracts.

A fundamental phytochemical description of Belamcanda sinensis and its components was given in the dissertation by Ms. A. Nenninger: (LMU München, 1997) entitled: "Phytochemische und pharmakologische Untersuchungen von Belamcanda sinensis, einer Arzneipflanze der TCM und anderer Irisarten".

With the medicaments of the invention, medicaments from Cimicifuga racemosa and Belamcanda sinensis and other Iridaceae and tectorigenin-based medicaments are for the first time available, which act as full estrogen receptor agonists in bones, in the cardiovascular system and in the brain.

Further advantages and features of the present invention become clear from the description of experimental data and by referring to the drawings, showing:

Fig. 1: a comparison of the organic and aqueous phases of Cimicifuga racemosa. Displacement graph of a representative estrogen receptor - ligand binding assay. The concentration of the start solution is 17.66 mg/ml, followed by dilutions 1:2, 1:4 etc. up to 1:64;

Fig. 2: serum LH prior to, and 2 hours after, intravenous injection of Belamcanda sinensis extract, E2 and vehicle. The Belamcanda sinensis extract has a similar capacity of lowering the elevated Serum LH levels as E2;

Fig. 3. effects of Cimicifuga racemosa and E2 on uterus weights (Fig. 3a) and LH levels in the blood (Fig. 3b) in ovariectomised rats after seven-day subcutaneous treatment; (mean values + SEM, n = 8, * = $p < 0.05$ vs. cremophor as vehicle);

Fig. 3a) uterus weights;

Fig. 3b) LH concentrations in the blood;

Fig 4a) effects of Cimicifuga racemosa and E2 in ovariectomised rats after seven-day subcutaneous treatment; (mean values + SEM, n = 8, * = $p < 0.05$ vs. cremophor as vehicle) on the expression of the mRNA for E2-receptor α in the preoptic region of the hypothalamus;

Fig 4 b) the expression of the mRNA for IGF1 and C3 in the uterus of ovariectomised rats after 7 days of subcutaneous administration; and

Fig 4 c) the expression of the mRNA for collagen 1 (Coll1) and osteocalcin in the bone of ovariectomised rats after 7 days of subcutaneous administration.

Experimental evidence for the estrogenic effect of Cimicifuga racemosa and Belamcanda sinensis

Selective estrogenic effect was demonstrated in stages in the course of a series of test systems of various degrees of complexity.

1. in-vitro experimentation

1.1 in-vitro experiments for Cimicifuga racemosa

Recognition of the estrogen-type structure of components by an antibody directed against 17-ß-estradiol (=E2) was shown in vitro.

The Cimicifuga racemosa extract was evaporated over residue. By phase distribution between dichloromethane and water, substances having different polarities were enriched. Binding affinities of the components of both phases were determined in vitro on estrogen receptors from pig's uterus. The cytosolic estrogen receptors from the pig uteri were isolated in accordance with standard procedures and used for the ligand displacement experiments.

Herein it was found that the estrogen-type structures e.g. from Cimicifuga racemosa are not hydrophilic in nature but lipophilic inasmuch as they may be extracted from the extract by means of an organic solvent. The substances present in the organically extracted phase bind about ten times more strongly to the antibody than the substances remaining in the aqueous phase.

The difference between the two phases is even greater in the estradiol receptor binding assay. The similarity of the binding substance with estradiol

must be high enough to enable a selective - competitive - interaction with the estradiol receptor to take place in a cell-free preparation. Inside this test system, the aqueous phase does not possess any activity, whereas the organic phase binds very strongly to the receptor.

The results are shown in Fig. 1.

1.2 in-vitro Belamcanda sinensis

It is known from other studies that extracts from Belamcanda sinensis also possess components which are recognised by an antibody against 17-ß-estradiol and bind to the 17-ß-estradiol receptor (cf. Nenninger loc.cit.). Surprisingly, however, the inventors of the present application have found that these extracts have different estrogenic effects on different organ systems, particularly that they do not have a uterotrophic effect.

2. in-vivo experiments: Evidence for the estrogenic effect on ovariectomised rat

Binding to the receptor E2 is very selective; it is, however, not possible to say whether the subsequent processes within the cell are promoted or inhibited, i.e. whether the substance is an agonist or an antagonist. This property can only be determined in suitable cellular systems or in the overall animal.

The ovariectomised rat is a recognised model for the post-menopausal woman in whom the endogenous estradiol production has subsided. As a result of the external supply of 17-ß-estradiol or of substances which have an estrogen-type effect, there occurs a restauration of estrogen-sensitive anatomical-morphological parameters, such as increase of the uterus weights and the occurrence of hornified cells, i.e. plaque epithelium cells at the vaginal epithelium, or hormonal changes such as lowering of the LH levels in the blood of the treated animals.

All experiments described hereinbelow were carried out with ovariectomised Sprague-Dawley rats (=ovx rats) having a weight between 240 and 280 g.

2.1 Single administration of Belamcanda sinensis

The onset of the effect of the estradiol-type effect of Belamcanda sinensis extract occurs very quickly. Even after a single i.v. administration of vehicle, estradiol and Belamcanda sinensis extract to ovx rats, pulsatility ceases both under E_2 and under Belamcanda sinensis. In the medicament value development, there result significant inhibitions of the serum LH levels, both in comparison with the previous values and in comparison with the cremophor-treated control animals. Cremophor is an emulsifier on the basis of polyethoxylated castor oil derivatives.

The results are represented in Fig. 2.

In the uterus of the animals six hours after injection of the Belamcanda sinensis extract, the expression of the uterine VEGF, IGF1 and C3 genes is not changed in comparison with the controls, whereas the estradiol injection brings about a clear increase of the gene expression of these three estrogen-regulated proteins. The constitutively expressed CCO gene was not significantly influenced by any one of these treatments.

These findings indicate that components of Belamcanda sinensis bring about an inhibition of the GnRH pulse generator in hypothalamic estrogen-receptive structures and thus have estrogen-agonistic effects. Hereby the hypophysary LH secretion is inhibited significantly both by components in Belamcanda sinensis and by estradiol. In contrast with estradiol, the components in Belamcanda sinensis do not have a uterotrophic effect. Estradiol significantly regulates the gene expression of VEGF, IGF1 and C3 upwardly, an effect which is not observed under Belamcanda sinensis.

Execution of the acute experiment on the effect of an i.v. injection of Belamcanda sinensis extract

24 rats (i.e. 8 animals/group) had a jugular vein catheter implanted under ether anesthesia on the day preceding the experiment. On the day of the experiment, 6 blood samples were taken at 10-min intervals. Immediately following taking of the 6th sample, 62.5 mg of the Belamcanda sinensis extract or 10 µg 17-ß-estradiol (E_2) or the solvent (5 %) cremophor in isotonic NaCl 1 ml), respectively, were injected intravenously, and blood

samples were taken for another 2 hours in 10-minute intervals. 6 hours after the intravenous administration, the animals were decapitated, blood was obtained and the uteri removed, weighed and deep-frozen in liquid nitrogen.

2.2 One-time administration of tectorigenin

Following a single administration of tectorigenin, the time development of influence on the LH levels in the blood and the estradiol-type immunoreactivity were determined. The concentration of tectorigenin in the blood of the animals, determined with the aid of E2-RIA, after 20 min corresponds to about 100 pg equivalent estradiol.

Tectorigenin triggers a rapid LH reduction. The kinetics of the LH reduction achieved under tectorigenin up to the time 60 min following i.v. administration precisely correspond to the one of estradiol, but then do not result in further reduction but slowly increases again.

Execution: OVX rats had catheters placed in the vena jugularis externa under ether anesthesia 24 hours before the beginning of the experiment, in accordance with the method of Harms and Ojeda (Harms PG; Ojeda SR: A rapid and simple procedure for chronic cannulation of the rat jugular vein. J. Appl. Physiol. (1974) 36: 391-392). The tube end was positioned in a skin pocket in the neck. In order not to have to touch the animals for obtaining the blood samples, the catheter was prolonged with the aid of a silicone tube. Catheter and tube were rinsed with Ringer solution containing 50 IU heparin/ml.

Blood samples of 100 µl each were drawn from the animals at 10-min intervals, and the withdrawn volume replaced with Ringer/heparin solution. After the 6th sample, 1.0 ml of the respective test solution was applied intravenously. As test solutions there were used: 2% cremophor (=vehicle solution), tectorigenin 7mg/ml vehicle, 17-ß-estradiol 10µg/ml vehicle. Blood was taken at ten-minute intervals through additional 140 min.

The blood samples thus obtained were filled into a 0.5 ml Eppendorf reaction vessel containing 10 µl heparin-Lösung (5000 IU/ml, Liquemin),

centrifuged for 10 min at 10 000 * g, and the plasma stored at -20°C until performance of the radioimmunoassays.

The RIAs for LH and Prolaktin are based on antisera, reference and iodisation preparations from NIH (Bethesda, Maryland, USA). The concentrations of estradiol and of the cross-reactive isoflavones were measured with the aid of an RIA from DPC, Bad Nauheim.

2.3 Effect of Belamcanda sinensis extract after administration through 7 days

The effects of repeated administration of estradiol, Belamcanda sinensis extract and vehicle on overall weight, uterus weight, hormone level and gene activation of uterus and bone were examined on ovariectomised rats after daily s.c. application through seven days.

The average body weights of the cremophor- and Belamcanda sinensis-treated animals do not differ, whereas the E_2-treated animals were significantly lighter. Neither do the uterus weights of the animals treated with cremophor and Belamcanda sinensis differ significantly, whereas the E_2-treatment more than tripled the uterus weights.

The serum LH levels in the Belamcanda sinensis-treated animals were reduced slightly, but significantly in comparison with the cremophor controls; reduction through estradiol was more marked.

In the uterine mRNA extract, estradiol significantly raised the gene expression of VEGF to 149% of the control value after a one-week treatment. Unter Belamcanda sinensis extract, expression was raised slightly but not significantly. Expression of the non estrogen-regulated constitutive genes for the cytochrome C oxidase (= CCO) was not influenced.

In extracts of the femur head, the collagen-1A1, osteocalcin, IGF1 and TGFß-mRNA expression was determined. Estradiol as well as Belamcanda sinensis significantly inhibited the expression of all 4 genes without having an influence on the constitutive CCO gene.

The different effects of estradiol and Belamcanda become very clear after the seven-day treatment. Belamcanda sinensis extract has an estradiol-

agonistic influence on the hypophysary LH secretion by inhibiting the GnRH impulse generator, and on the gene expression of four estrogen-regulated genes in the bone. In contrast, there is no estrogenic effect on the uterus: neither the uterus weight nor the estrogen-regulated VEGF gene are influenced by the Belamcanda sinensis extract. In contrast, estradiol brings about ballooning of the uterus and an activation of the VEGF gene.

Execution of the subacute test on the effect of daily s.c. injection through 7 days:

8 animals each per test group (24 altogether) were daily injected subcutaneously between 8:00 and 9:00 a.m. with 62.5 mg Belamcanda sinensis extract and 10 µg estradiol or the solvent (cremophor 5%, 1 ml), respectively. 6 h after the last application, the animals were decapitated and from every animal the aorta, the uterus and the left femur head were removed, cleaned, and frozen in liquid nitrogen.

In the blood samples, LH and the estradiol immunoreactivity were determined.

2.4 Repeated administration of Cimicifuga racemosa

14 days following ovarectomy at the earliest, the animals have the respective test substance injected subcutaneously in a dose of 62.5 mg Cimicifuga racemosa/rat or 8 µg estradiol/rat once daily in the morning over a period of 7 days. Both substances were dissolved in 5% cremophor, the control animals only received the vehicle.

Following decapitation of the animals, brains, uterus and femur were prepared for mRNA-recovery. The LH concentration in the blood of the animals was determined by means of RIA. The expression of the estrogen-regulated genes in the above identified organs was determined by means of semi-quantitative RT-PCR.

The uteri of the estradiol-treated animals have more than three times the weight of those of the animals treated with Cimicifuga racemosa and vehicle which basically do not differ in their mean values. This means that the components of Cimicifuga racemosa have no influence on the uterus of the animals. This is also true for the vagina, where no hornification of the

epithelium tissue occurs in the animals treated with Cimicifuga racemosa and vehicle, quite contrary to the estradiol-treated animals.

The LH levels of the vehicle-treated animals remain high, however are lowered significantly both by estradiol and Cimicifuga racemosa.

The results are shown in Figs. 3a) and 3b).

Uterus weights (wet)

	Cremophor [control]	Cimicifuga racemosa	E2
Number animals	8	8	8
Mean values [mg]	185.6	192.3	702.1
SD	18.81	22.53	194.97
SEM	6.65	7.97	68.92

LH concentrations in the blood

	Cremophor [control]	Cimicifuga racemosa	E2
Number animals	8	8	8
Mean values [ng/ml]	16.9	12.5	7.83
SD	3.99	3.4	5.57
SEM	1.41	1.2	1.97

As another marker for the estrogene effect, the activation of mRNA of estrogen-induceable proteines was measured. What was measured here was tissue from uterus, from bone tissue (femur) and from the preoptic region of the hypothalamus.

In the hypothalamus, both Cimicifuga racemosa and E2 stimulate the expression of the mRNA for the estrogen receptor α (Fig 4a). In the bone tissue, too, Cimicifuga racemosa behaves like an estrogen and reduces, in analogy with estradiol, the expression of the mRNA for the bone-specific collagen 1 and for osteocalcin genes (Fig 4b).

In contrast, no effect of Cimicifuga racemosa on estrogen-regulated genes in the uterus is observed. Only estradiol increases the mRNA for IGF1 and complement factor C3 (Fig 4c).

These findings prove that the components from Cimicifuga racemosa selectively act on single organs: the extract acts estrogenically in the hypothalamus (expression of the E2 receptor α, liberation of LH) and on the bone, proven by the expression of the genes for collagen 1 and osteocalcin. Other than estradiol, however, Cimicifuga racemosa does not have an effect on the uterus, as the absence of an effect on the uterus weights and the expression of the genes for IGF1 and C3 shows.

By the experiments carried out in vitro and in vivo, it could be demonstrated that Cimicifuga racemosa and Belamcanda sinensis extracts exert an estrogenic effect. Surprisingly it was found that the extracts from the named drugs act organoselectively on central nervous system, bone and vessels, but not on the uterus, and are thus excellently suited for the prophylaxis and therapy of estrogen deficiency without having a negative influence on the endometrium.

Identical effects are achieved by the tectorigenin contained in Belamcanda.

Thus for the first time medicaments having an estrogen-type effect, however without a uterotrophic effect, are available.

The like medicaments may be used for the treatment and/or prophylaxis of cardiovascular diseases, particularly atherosclerosis, osteoporosis, and of peri- and post-menopausal psychovegetative disorders such as, e.g., hot flushes.

Among types of application, oral, intravenous and subcutaneous application are prominent.

Amended Claims

1. Use of extracts from Iridaceae for producing an estrogen-type, organoselective medicament having no uterotrophic effect or one that is at least negligible, under the proviso that Belamcanda chinensis extract is not used if the medicament is used for alleviating peri-menopausal and post-menopausal disorders.

2. Use in accordance with claim 1, characterised in that the extracts are produced from Belamcanda chinensis.

3. Use of extracts from Cimicifuga racemosa for producing an estrogen-type, organoselective medicament having no uterotrophic effect or one that is at least negligible, under the proviso that the medicament is not used for alleviating peri-menopausal and post-menopausal disorders and dysmenorrhea.

4. Use of extracts containing tectorigenin and/or tectorigenin glycoside, with the exception of extracts from Iridaceae, or extracts enriched with tectorigenin and/or tectorigenin glycoside for producing an estrogen-type, organoselective medicament having no uterotrophic effect or one that is at least negligible.

5. Use in accordance with any one of claims 1 to 3, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of cardiovascular diseases, particularly atherosclerosis.

6. Use in accordance with any one of claims 1 to 3, characterised in that the extract serves for producing a ready-formulated medicament for the selective treatment and/or prophylaxis of osteoporosis.

7. Use in accordance with any one of claims 1 to 4, characterised in that the extract serves for producing a ready-formulated medicament for the

selective treatment and/or prophylaxis of climacteric disorders, particularly for preventing or alleviating hot flushes.

8. Use of tectorigenin and/or its glycosides for producing an estrogen-type, organoselective medicament having no uterotrophic effect or one that is at least negligible.

9. Use in accordance with claim 8, characterised in that it is a medicament for the selective treatment and/or prophylaxis of cardiovascular diseases, particularly atherosclerosis;

osteoporosis; and climacteric disorders, particularly for preventing or alleviating hot flushes.

Abstract of the Disclosure

Use of extracts from Iridaceae and Cimicifuga racemosa and of tectorigenin as an estrogen-type, organoselective medicament having no uterotrophic effect

The present invention relates to the use of extracts from Iridaceae and from Cimicifuga racemosa, and of tectorigenin as an estrogen-type, organoselective medicament for the selective treatment and/or prophylaxis of cardiovascular diseases, in particular atherosclerose, osteoporose and climacteric disorders, e.g. for preventing or alleviating hot flushes. Uterotrophic effects are practically not observed.

(Fig. 2)

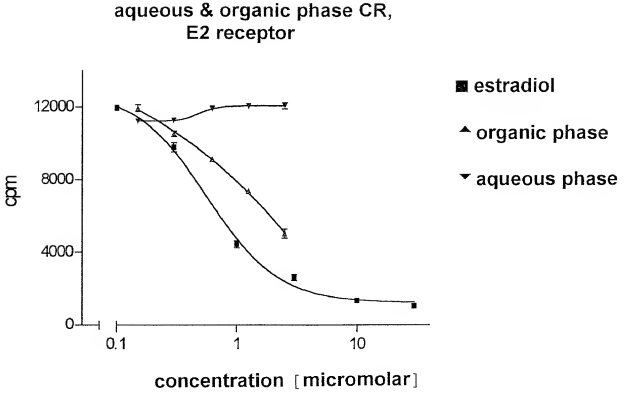

aqueous & organic phase CR,
E2 receptor
12000
8000
4000
0
cpm
0.1
1
10
concentration [micromolar]
estradiol
organic phase
aqueous phase

Fig. 1

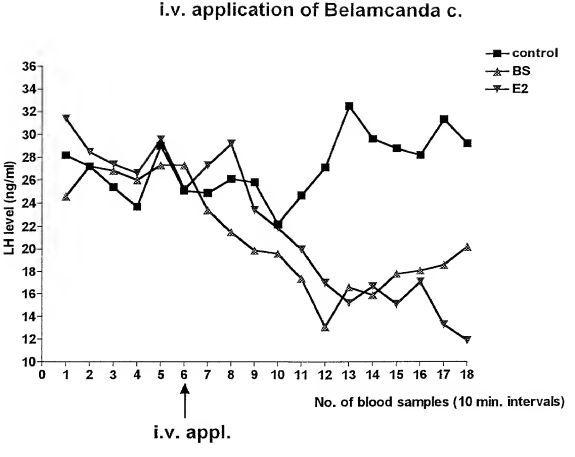
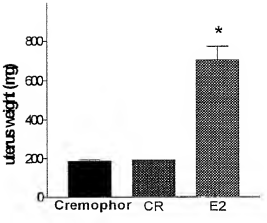
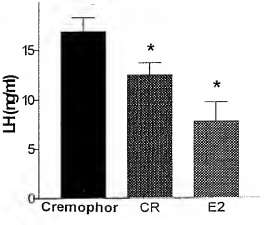

i.v. application of Belamcanda c.
control
BS
E2
LH level (ng/ml)
36
34
32
30
28
26
24
22
20
18
16
14
12
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
No. of blood samples (10 min. intervals)
i.v. appl.

Fig. 2



3a) uterus weights

Fig. 3a



3b) LH concentrations in the blood

Fig. 3b

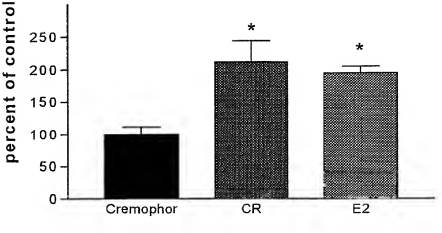


Fig. 4a

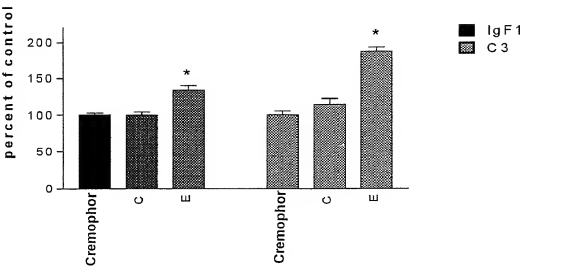

percent of control
200
150
100
50
0
*
*
IgF1
C3
Cremophor
C
E
Cremophor
C
E

Fig. 4b

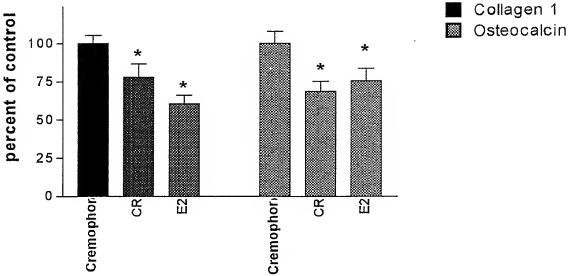

percent of control
100
75
50
25
0
*
*
*
*
Collagen 1
Osteocalcin
Cremophor
CR
E2
Cremophor
CR
E2

Fig. 4c

PTO/SB/103 (8-96
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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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Declaration and Power of Attorney for Patent Application
Erklärung für Patentanmeldungen mit Vollmacht

German Language Declaration

Als nachstehend benannter Erfinder erkläre ich hiermit an Eides Statt:

As a below named inventor, I hereby declare that:

daß mein Wohnsitz, meine Postanschrift und meine Staatsangehörigkeit den im nachstehenden nach meinem Namen aufgeführten Angaben entsprechen, daß ich nach bestem Wissen der ursprüngliche, erste und alleinige Erfinder (falls nachstehend nur ein Name angegeben ist) oder ein ursprünglicher, erster und Miterfinder (falls nachstehend mehrere Namen aufgeführt sind) des Gegenstandes bin, für den dieser Antrag gestellt wird und für den ein Patent für die Erfindung mit folgendem Titel beantragt wird:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

UTILIZATION OF EXTRACTS FROM IRIS PLANTS, CIMICIFUGA RACEMOSA AND TECTORIGENIN AS AN ESTROGEN-LIKE ORGAN -SELECTIVE MEDICAMENT WITHOUT UTEROTROPIC EFFECTS

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the specification of which is attached hereto unless the following box is checked:

☐ wurde angemeldet am _____ unter der US-Anmeldenummer oder unter der Internationalen Anmeldenummer im Rahmen des Vertrags über die Zusammenarbeit auf dem Gebiet des Patentwesens (PCT) _____ und am _____ abgeändert (falls zutreffend).

☒ was filed on 19 MARCH 1999 as United States Application Number or PCT International Application Number PCT/EP99/01860 and was amended on June 7, 2000 (if applicable).

Ich bestätige hiermit, daß ich den Inhalt der oben angegebenen Patentanmeldung, einschließlich der Ansprüche, die eventuell durch einen oben erwähnten Zusatzantrag abgeändert wurde, durchgesehen und verstanden habe.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Ich erkenne meine Pflicht zur Offenbarung jeglicher Informationen an, die zur Prüfung der Patentfähigkeit in Einklang mit Titel 37, Code of Federal Regulations, § 1.56 von Belang sind.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

PTO/SB/103 (8-96)
Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

German Language Declaration

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Prior Foreign Applications (Frühere ausländische Anmeldungen)			Priority Not Claimed Priorität nicht beansprucht
19812204.7 (Number) (Nummer)	DE (Country) (Land)	March 19, 1998 (Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)	☐
(Number) (Nummer)	(Country) (Land)	(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)	☐

Ich beanspruche hiermit Prioritätsvorteile unter Title 35, US-Code, §119(e) aller US-Hilfsanmeldungen wie unten aufgezählt.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)
(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)

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(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)	(Status) (patented, pending, abandoned) (Status) (patentiert, schwebend, aufgegeben)
(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)	(Status) (patented, pending, abandoned) (Status) (patentiert, schwebend, aufgegeben)

Ich erkläre hiermit, daß alle in der vorliegenden Erklärung von mir gemachten Angaben nach bestem Wissen und Gewissen der Wahrheit entsprechen, und ferner daß ich diese eidesstattliche Erklärung in Kenntnis dessen ablege, daß wissentlich und vorsätzlich falsche Angaben oder dergleichen gemäß § 1001, Title 18 des US-Code strafbar sind und mit Geldstrafe und/oder Gefängnis bestraft werden können und daß derartige wissentlich und vorsätzlich falsche Angaben die Rechtswirksamkeit der vorliegenden Patentanmeldung oder eines aufgrund deren erteilten Patentes gefährden können.

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German Language Declaration

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

Postanschrift:

Send Correspondence to:

Telefonische Auskünfte: *(Name und Telefonnummer)*

Direct Telephone Calls to: *(name and telephone number)*

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Unterschrift des Erfinders Datum	Inventor's signature Date Dr. Wolfgang Wuttke 12.9.00
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Postanschrift	Post Office Address Domane 8 37120 Bovenden Germany
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Unterschrift des zweiten Erfinders Datum	Second Inventor's signature Date Dr. H. Jarry 12.09.2000
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(Supply similar information and signature for third and subsequent joint inventors.)

See attached page 4 for signatures of subsequent joint inventors VOLKER CHRISTOFFEL, BARBARA SPENGLER and MICHAEL POPP

Signatures by Subsequent Joint Inventors
on Declaration for Patent Application entitled
UTILIZATION OF EXTRACTS FROM IRIS PLANTS,
CIMICIFUGA RACEMOSA AND TECTORIGENIN
AS AN ESTROGEN-LIKE ORGAN-SELECTIVE
MEDICAMENT WITHOUT UTEROTROPIC
EFFECTS, the specification of which was filed on 19 March 1999
as PCT International Application No. PCT/EP99/01860:

300

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Third Joint Inventor's Signature — Date

V. Christoffel — 28.08.2000

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Barbara Spengler — 28.08.2000

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Full Name of fifth joint inventor: Michael POPP
Third Joint Inventor's Signature — Date

M. Popp — 28.8.2000

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

POWER OF ATTORNEY

Docket No.
WINTE 045244

Name of Applicant: **Inventors: Wolfgang Wuttke, et al.**
Address of Applicant: **Assignee: Bionorica Arzneimittel GmbH**
Kerschensteinerstrasse 11-15
DE-92318 Neumarkt
GERMANY
Title: **UTILIZATION OF EXTRACTS FROM IRIS PLANTS, CIMICIFUGA RACEMOSA AND TECTORIGENIN AS AN ESTROGEN-LIKE ORGAN-SELECTIVE, etc.**
Serial No., if Any:
Filed:

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The Assistant Commissioner for Patents
Washington, D.C. 20231

Honorable Sir:
I hereby appoint:

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Jeffrey F. Craft, Esq., Reg. No. 30,044
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Dated: Neumarkt, 28.07.2000